ILOs

• Explain the principals of the techniques used for genomic DNA Quality assessment
  ➢ DNA Quantification
  ➢ DNA Purity
  ➢ DNA integrity
• Apply relevant techniques to QC our DNA extracts
• Explain the Principals of DNA concentration calculations
• Apply this on our DNA extract to calculate the amounts needed per sample to pool the sequencing library at equimolar concentrations
Purity
- Nanodrop™

Quantity
- Qubit™
- Nanodrop™
- TapeStation™/Bioanalyser™

Integrity
- TapeStation™/Bioanalyser™
- Gel Electrophoresis
Purity

Contaminants
- Proteins
- RNA
- Chemical impurities e.g. detergents, denaturants, chelating agents
- high concentration of salts (affect efficiency of enzymatic steps)

Poor Sequencing Library
NanoDrop™ is a spectrophotometer that can be used to quantify the DNA and protein content in a 1-2 µl sample.

RNA is a common contaminant in genomic DNA extracts.

Nanodrop cannot distinguish between DNA and RNA very well;

Hence less accurate for quantification of DNA for library preparation purposes.

Detection limit is 2ng/µL
Nanodrop™ Microvolume UV-Vis Spectrophotometer

- Nucleic acids (such as DNA) absorb light at the 260 nm
- Proteins absorb light at 280 nm
- Phenolic compounds, EDTA, Carbohydrates read at 230 nm

Measure the purity of DNA extracts through measuring the
✓ A260/A280 ratio (recommended values ~1.8)
  • <1.8 -> High protein content and other impurities
  • (poor library preparation)
  • >2 -> High RNA content
✓ A260/A230 recommended value (2.0 – 2.2)
  • A260/A230 significantly <2 indicates the presence of contaminants ->
    purification or PCR amplification (in library prep)
Nanodrop™ Microvolume UV-Vis Spectrophotometer
Purity
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High Specificity of Qubit dsDNA Assay Kits provides accurate quantification of Nucleic acids.

Target >53 ng/µl

DNA Kits
- High sensitivity (HS) kit 0.01-100 ng/uL.
- Broad range (BR) kit 0.2-4000 ng/uL.

Components of the kit
- Qubit® dsDNA HS Reagent (Component A)
- Qubit® dsDNA HS Buffer (Component B)
- Qubit® dsDNA HS Standard #1 (Component C)
- Qubit® dsDNA HS Standard #2 (Component D)

Qubit® Assay tubes
Qubit™ Fluorometer

Prepare the Assay Tubes* according to the table below.

<table>
<thead>
<tr>
<th></th>
<th>Standard Assay Tubes</th>
<th>User Sample Assay Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of <strong>Working Solution</strong> (from step 2) to add</td>
<td>190 μL</td>
<td>180–199 μL</td>
</tr>
<tr>
<td>Volume of <strong>Standard</strong> (from kit) to add</td>
<td>10 μL</td>
<td>—</td>
</tr>
<tr>
<td>Volume of <strong>User Sample</strong> to add</td>
<td>—</td>
<td>1–20 μL</td>
</tr>
<tr>
<td>Total Volume in each Assay Tube</td>
<td>200 μL</td>
<td>200 μL</td>
</tr>
</tbody>
</table>

Dilution Factor

- 1μL -> 200x
- 2μL -> 100x
Qubit™ Fluorometer

Ensure all reagents are at room temperature

Qubit™ Reagent

1 × n μL

Qubit™ Working Solution

199 × n μL

Qubit™ Buffer

190 μL

Final volume is 200 μL

Standards from kit

10 μL

10 μL

User Samples

1–20 μL

1–20 μL

1–20 μL

Final volume is 200 μL

Vortex all assay tubes for 2–3 seconds

Incubate at room temperature for 2 minutes (15 minutes for Qubit™ protein assay)

Read tubes in Qubit® 2.0 Fluorometer

* where n = number of Standards plus number of Samples

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- Purity
  - Nanodrop™

- Quantity
  - Qubit™
  - Nanodrop™
  - TapeStation™/Bioanalyser™

- Integrity
  - TapeStation™/Bioanalyser™
  - Gel Electrophoresis
Integrity

Mwt

Degradation
Gel Electrophoresis Principle

DNA is separated based on their migration rate in a gel matrix (e.g. Agarose) under the influence of electric field.

DNA carry charge?
What are Factors?

Migration Rate

• Size of the DNA fragment
• Voltage of Electric field
• Porosity of the gel matrix (Resistance)

Resolution
DNA Ladder + Tracking Dye
Loading Dye 6x Dilution 1:5
Tris/Borate/EDTA buffer (TBE buffer) Prepare 1x or 0.5x TBE buffer
Agarose (0.7%) (? - 100mL) (? - 50 mL) TBE buffer

GelRed® (Biotium) or Ethidium Bromide
Gel Electrophoresis

- Conventional agarose gel electrophoresis employs a static field and can resolve DNA fragments up to 50 kb
- For Resolution of larger Fragments: Pulsed Gel Electrophoresis (PFGE) is more useful

https://www.youtube.com/watch?v=k_QAXLGuQ5w
Diffuse Smearing

Concentration

a

Cycler 1.25 ng, Cycler negative, Chip 0.125 ng, Chip 0.0125 ng, Chip 0.00125 ng, Chip negative

b

223 bp amplicon_on-chip

Normalised band intensity

DNA template concentration

0.125 ng, 0.0125 ng, 0.00125 ng

Georgia Kaprou et al. 2020. Towards PCB-Based Miniaturized Thermocyclers for DNA Amplification
Agilent Bioanalyzer/ TapeStation 1µL Sample

- Agilent 2100 Bioanalyzer instrument
- Chips disposable
- **Kits**
  - **Agilent DNA 12000** kits are designed for the sizing and quantitation of double-stranded DNA fragments from 100 to 12000 bp.
  - **Agilent DNA 7500** 100 – 7500 bp
  - Can analyse 12 samples/chip, chip can not be reused.

Agilent 4200 or 2200 TapeStation
Credit card size Screen Tape 16 Samples/ Card
Can run up to 95 samples in a plate
The unused lanes in Card is reusable -> fixed cost per sample

**Kits** (Reagents + Screen tape):
- **Genomic DNA kit** : Broad Range 100-60,000 bp
- Agilent D1000 Kit/Agilent HS D1000 up to 1000 bp
- Agilent D5000 Kit/Agilent HS D5000 up to 5000 bp
Bioanalyzer
TapeStration
Card Size
Screen Tape

### Kit Components (Genomic DNA ScreenTape Assay)

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Name</th>
<th>Color</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>5067-5365</td>
<td>Genomic DNA ScreenTape</td>
<td></td>
<td>7 ScreenTape devices</td>
</tr>
<tr>
<td>5067-5366</td>
<td>Genomic DNA Reagents</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Genomic DNA Ladder</td>
<td></td>
<td>25 µL</td>
</tr>
<tr>
<td></td>
<td>- Genomic DNA Sample Buffer</td>
<td></td>
<td>1356 µL</td>
</tr>
</tbody>
</table>
TapeStation™ Results, Which is Better?

A

B
Purity
- Nanodrop™

Quantity
- Qubit™
- Nanodrop™
- TapeStation/Bioanalyser

Integrity
- TapeStation/Bioanalyser
- Gel Electrophoresis
What to aim for?

Purity
- Nanodrop™
  - A260/A280 1.8 - <2.00
  - An Estimate of the concentration

Quantity
- Qubit™
  - Concentration >53 ng/μL

Integrity
- TapeStation/Bioanalyser
- Gel Electrophoresis
  - High Mwt genomic DNA >12 kb
  - No Degradation (Smearing, High sharp peak)
Concentration = \( \frac{\text{weight}}{\text{Volume}} \)

Molarity = \( \frac{\text{weight} (g)}{\text{Mwt}} \times \frac{1}{\text{volume} (Lt)} \)

Mwt of DNA Fragment = size of a fragment (TapeStation) \times \text{average Mwt of bp} (660 \text{ Da})

A mole of anything has Avogadro’s number \( 6.022 \times 10^{23} \) of this thing.

Rule: Pooling the Sequencing library at equal equimolar ratio.
What does this practically mean?
Any Questions?